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NITRIC OXIDE IN THE PENIS: PHYSIOLOGY AND PATHOLOGY

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ABSTRACT

Purpose: The significance of nitric oxide in the physiology of the penis was evaluated, including its role in pathophysiological mechanisms and pathological consequences involving this organ.

Materials and Methods: Animal and human studies pertaining to nitric oxide in the penis were reviewed and analyzed in the context of current descriptions of the molecular biology and physiological effects of this chemical.

Results: Potential sources of nitric oxide in the penis include neurons, sinusoidal endothelium and corporeal smooth muscle cells. Nitric oxide is perceived to exert a host of functional roles by binding with specific molecular targets. Its synthesis and action in the penis are influenced by many different regulatory factors.

Conclusions: Nitric oxide exerts a significant role in the physiology of the penis, operating chiefly as the principal mediator of erectile function. Alterations in the biology of nitric oxide likely account for various forms of erectile dysfunction. The diverse physiological roles of nitric oxide suggest that it may also directly contribute to or cause pathological consequences involving the penis.

KEY WORDS: penis, penile induration, pathology, physiology, nitric oxide

Recent advances in the functional anatomy, vascular smooth muscle physiology, neurophysiology and pharmacology of penile erection have fostered a greatly improved contemporary understanding of the erectile process and the pathophysiology associated with erectile dysfunction. Penile erection is a hemodynamic process, involving increased arterial inflow and restricted venous outflow from the penis, coordinated with corpus cavernous smooth muscle relaxation.[1] The process is generally accepted to be under neuroregulatory control and involves the cholinergic, adrenergic and nonadrenergic noncholinergic neuroeffector systems. However, biochemical substances locally released from the endothelial or smooth muscle components of the erectile tissue may also cooperate in this function.

Since neither cholinergic nor adrenergic mechanisms can fully explain the regulatory events involved in erectile function, the concept of a nonadrenergic noncholinergic inhibitory system has been invoked. Diverse mediators have been proposed to function as nonadrenergic noncholinergic neurotransmitters, including neuropeptides (vasoactive intestinal peptide, calcitonin gene-related peptide, substance P), purines (adenosine, adenosine triphosphate) and other factors, including decarboxylated amino acids, histamine, serotonin, prostaglandins and bradykinin. However, convincing evidence supporting any of these candidates as the essential mediator of penile erection has been lacking.

The search for the elusive agent of penile erection has recently led to nitric oxide, a gaseous messenger molecule, for this role. Current research suggests that this chemical provides the critical component of an unusual regulatory system that is responsible for effecting smooth muscle relaxation during erection. In addition to this function, nitric oxide by virtue of its diverse biochemical properties may exert other important physiological effects in the penis as well. This review examines the primary role of nitric oxide in the penis as the principal mediator of penile erection and explores other possible mechanisms whereby nitric oxide may determine function in the penis. Emphasis is given to the sources and synthesis of nitric oxide, its mechanisms of action and its interaction with other biochemical or mechanical factors that may exert a regulatory influence over nitric oxide synthesis and action in the penis.

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NITRIC OXIDE AS A BIOLOGICAL EFFECTOR

The prospect that nitric oxide could fulfill the elusive role as mediator of penile erection extends from investigative groundwork laid by several disciplines during recent years. Presently, nitric oxide is understood to account for diverse biological functions, including immune system responses, vascular regulatory effects and neurotransmission.[2–6] Thus, whereas this chemical had formerly been inferred to be a potentially toxic, free radical gas, it now is implicated in diverse physiological activities as well.

A primary biochemical role for nitric oxide is to stimulate the increased intracellular production of the second messenger molecule, 3′,5′-cyclic guanosine monophosphate (cGMP).[2–6] Nitric oxide accounts for this effect by binding to the heme moiety of guanylate cyclase following its local production and diffusion. This step activates guanylate cyclase, which then catalyzes the formation of cGMP from guanosine 5′-triphosphate. Much like the cyclic nucleotide 3′,5′-cyclic adenosine monophosphate, cGMP modulates the activities of specific protein kinases and thereby regulates diverse intracellular functions. Additional biochemical roles for nitric oxide include glycolytic enzyme interactions, reactions with various compounds (thiols, heme groups and iron sulphur centers) to generate long-term storage forms of nitric oxide (nitrosylation) and superoxide formation.[2–6]

Nitric oxide is synthesized as a by-product of the catalytic conversion of L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS). A family of NOS enzymes exists and consists of constitutive and inducible NOS isoforms that differ according to molecular, biochemical and pharmacological properties.[5,6] The constitutive NOS isoforms commonly exist in endothelial cells and neurons, and are activated in the presence of calcium, the calcium-binding protein calmodulin, oxygen and reduced nicotinamide adenine dinucleotide phosphate, whereas arginine derivatives typically inhibit their catalytic activity. The constitutive isoforms, while always

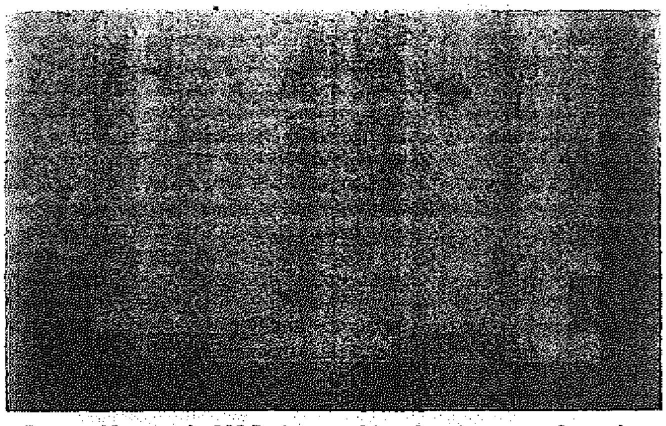

FIG. 1. Neuronal NOS immunohistochemistry performed on cross-section through proximal penis. NOS containing nerve bundles are noted at periphery of deep cavernous artery and coursing within cavernous tissue. Reduced from ×100.

present, are inactive until intracellular calcium levels increase, calmodulin binds to calcium, and the calcium-calmodulin complex binds to and activates NOS. Nitric oxide is then synthesized and released in small amounts until calcium levels decrease; this periodic production of nitric oxide transmits signals. In contrast, inducible NOS is typically associated with macrophages and other cells of immune function using tetrahydrobiopterin as its main cofactor. This enzyme is expressed when these cells are activated by specific cytokines and its regulation involves transcriptional factors. The large, continuous amounts of nitric oxide always synthesized by inducible NOS in these cells are pathogenic to surrounding cells, bacteria and parasites.

NITRIC OXIDE RELAXES ERECTILE TISSUE

A fundamental principle of erection physiology is that the corporeal smooth musculature of the penis must undergo relaxation for physiological penile erection to occur. Accordingly, corporeal smooth muscle tone has long been perceived to be under regulatory control involving 1 or a combination of mechanisms. The premise that nitric oxide could modulate corporeal smooth muscle function concurs with its original description as endothelium-derived relaxing factor (EDRF): this substance was discovered in vasculature to derive from endothelial cells and induce vascular smooth muscle relaxation.[7,8]

Support for nitric oxide as a regulator of corporeal smooth muscle relaxation has derived from biochemical, histochemical and physiological erection studies. Organ bath studies demonstrating nitric oxide relaxant effects initially in isolated rodent anococcygeal and bovine retractor penile muscles, and subsequently in human and animal corpus cavernous and spongiosal tissue specimens provided early evidence for this role.[9–18] The strategy among these investigations was common: direct application of nitric oxide or its substrate, L-arginine, caused tissue relaxation, resembling electrical (neurogenic) stimulation effects, and administration of agents that blocked nitric oxide synthesis or its mode of action abolished relaxant effects, whether elicited by pharmacological or nerve stimulation. In vivo animal paradigms of penile erection in which similar methodologies were used further established the regulatory role of nitric oxide in physiological penile erection.[19–25] Biochemical measurements of NOS activity and localizations of NOS by immunoblot analysis and immunohistochemistry in human and animal studies using neuronal NOS specific antibodies have strengthened the basis for NOS and the nitric oxide regulatory pathway in the penis (figs. 1 and 2).[19,26–31] NOS has also been localized to lumbosacral pathways that are implicated in governing erectile function.[29,32]

At present, much evidence supports the concept that nitric oxide derives from the autonomic innervation of the penis and locally operates as a postganglionic neurotransmitter of nonadrenergic noncholinergic mediated penile erection. Upon its synthesis and release from nerve terminals within the erectile tissue of the penis (via neurogenic pathways), nitric oxide diffuses to neighboring vascular and trabecular smooth musculature of the penis whereby it activates guanylate cyclase present in smooth muscle cells to produce cGMP. The increased intracellular accumulation of cGMP is perceived to cause corporeal smooth muscle relaxation via biochemical cascade. A putative mechanism involves cGMP dependent protein kinase dephosphorylation of myosin light chains in corporeal smooth muscle cells (directly or as a consequence of lowering intracellular calcium stores). Nitric oxide via effects on transcellular ion fluxes could also influence the contractile state of corporeal smooth musculature. Recent research suggests that nitric oxide activates sodium/potassium-adenosine triphosphatase in human and rabbit corporeal smooth musculature,[33,34] as well as a potassium conductive membrane hyperpolarization pathway in rabbit corporeal smooth muscle cells.[35]

Penile constituents other than neurons may be considered to generate nitric oxide as well. Transgenic mice lacking the neuronal NOS gene preserve erectile function,[36] suggesting the collaborative or compensatory effects of other sources of nitric oxide (or of other mediators). Vascular and sinusoidal endothelium produces nitric oxide, supported by recent immunolocalization studies directly establishing the endothelial NOS isoform in penises of neuronal NOS deficient mice as well as normal animals.[37] Alternatively, the smooth muscle component of erectile tissue could conceivably generate nitric oxide, insofar as recent investigations have verified the in vitro expression of inducible NOS in rat corporeal smooth muscle cells.[38] However, whether and to what extent these isoforms sufficiently participate in physiological erectile function await further elucidation.

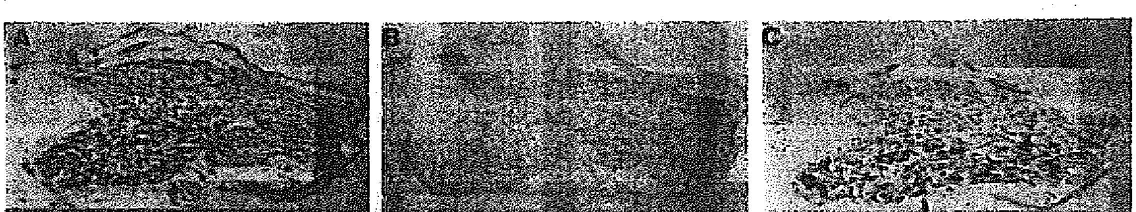

FIG. 2. Serial tissue sections of histochemical localization of NOS in major pelvic ganglion of male rat. *A*, immunohistochemistry with purified antineuronal NOS antibody. *B*, immunohistochemistry with pre-immune serum (negative control). *C*, nicotinamide adenine dinucleotide phosphate diaphorase histochemistry (positive control). Regional distribution of ganglion cell bodies selectively exhibits NOS immunoreactivity. Reduced from ×100.

NITRIC OXIDE REGULATORY INFLUENCES IN THE PENIS

Ongoing investigations studying the role of nitric oxide in the penis have also focused on its interaction with other biochemical factors and effector systems that operate in the penis (fig. 3). Various factors have been reported to interfere with the biochemical formation or action of nitric oxide, including the presence of oxyhemoglobin or superoxide anion, hypoxia, low intracellular calcium stores and extreme acid-base conditions.[6,39] NOS gene expression may be influenced by cofactor requirements, covalent modification mechanisms and the interaction of diverse signaling pathways.[6] Other regulatory influences include certain growth factors, cytokines and transcription factors.[6] Mechanical factors, such as local blood flow phenomena, have been shown in experimental hemodynamic models to determine endothelial NOS gene expression involving an endothelial shear stress response element located on the 5'-flanking region of the endothelial NOS gene. Since somatic nerve damage in the rat is associated with increases in neuronal NOS messenger ribonucleic acid levels in ipsilateral dorsal root ganglia, a mechanical mechanism may also be implicated in affecting neuronal NOS expression. Nitric oxide itself has been postulated to exert a direct feedback inhibition of NOS activity by interacting with the heme moiety of the enzyme.

Direct evidence relating nitric oxide regulatory factors to effects in the penis has come from several studies. Oxygen tension has a major role in nitric oxide mediated penile erections since low oxygen states inhibit nitric oxide synthesis.[40] The presence of advanced glycosylation end products adversely affects nitric oxide mediated rabbit and human corporeal tissue relaxation.[41] On the other hand, normally high levels of tissue superoxide dismutase in bovine retractor penis smooth muscle protect nitric oxide from destruction by superoxide anions.[42]

The influence of androgens on the nitric oxide pathway in the penis has been a subject of recent intense study.[43–45] Androgen deprivation reduces NOS content, activity and erectile responses in the rat penis, whereas its replacement in castrated rats restores these effects. These latter consequences are attributed to dihydrotestosterone as the active androgen[45] but the precise central or peripheral mechanism that preserves this function remains to be fully delineated. Systemic androgen levels also affect NOS activities assayed in electrically stimulated erect rat penises.[46]

Nitric oxide effects in the penis may also be induced or modulated by cotransmitters. Acetylcholine and bradykinin stimulate endothelial NOS pathways to generate nitric oxide mediated smooth muscle relaxation of human and rabbit corporeal tissue.[47–49] Vasoactive intestinal peptide may also exert a relaxant effect on corporeal smooth musculature via the nitric oxide-cGMP pathway, in support of its role as an ancillary nonadrenergic noncholinergic neurotransmitter of penile erection. Vasoactive intestinal peptide has been found to co-localize with NOS in human and rat penile neurons,[50,51] and inhibitors of NOS or guanylate cyclase attenuate vasoactive intestinal peptide induced relaxation of rabbit corporeal tissue.[48,52]

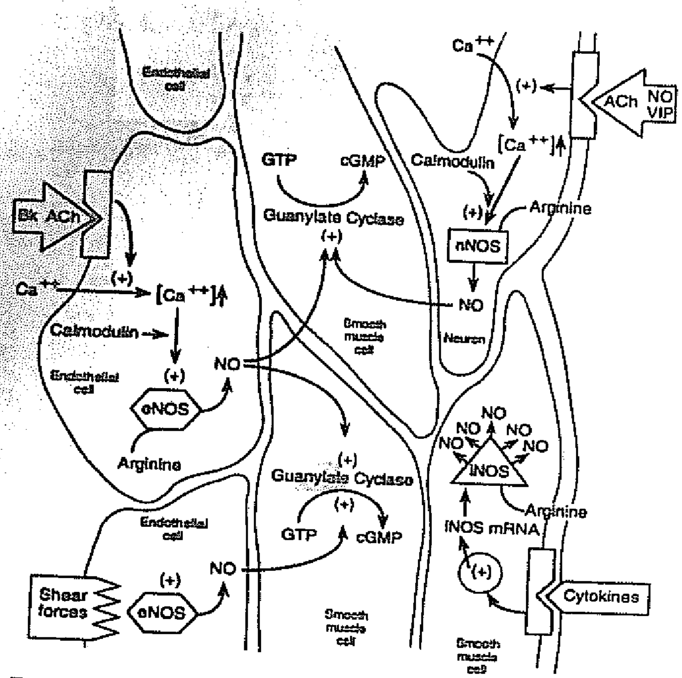

FIG. 3. Proposed mechanisms for nitric oxide (*NO*) synthesis, regulation and action in penis. Nitric oxide is constitutively formed from its precursor, L-arginine, in endothelial cells and neurons by catalytic action of endothelial NOS (*eNOS*) and neuronal NOS (*nNOS*), respectively. Whereas messenger molecules commonly activate these enzymes by signaling influx of calcium and its binding with calmodulin, other biochemical or mechanical factors may interact with this process influencing production of nitric oxide. Once synthesized, nitric oxide diffuses to local smooth muscle cells where it primarily activates guanylate cyclase to convert 5'-guanosine triphosphate (*GTP*) to 3',5'-cyclic guanosine monophosphate (*cGMP*). Smooth muscle cells represent another source of nitric oxide but they appear to require cytokine stimulation of inducible NOS (*iNOS*) expression. *Bk*, bradykinin. *ACh*, acetylcholine. *VIP*, vasoactive intestinal peptide.

NITRIC OXIDE IN THE PATHOPHYSIOLOGY OF THE PENIS

In view of the apparent role of nitric oxide as the key chemical element necessary for penile erection, investigators have begun to explore the relationship between medical conditions associated with erectile dysfunction and altered nitric oxide synthesis or action in the penis. Diabetes mellitus has been associated with impaired NOS dependent erectile mechanisms, presumably related to the antiproliferative effects of advanced glycosylation end products on nitric oxide formation.[41] Hypercholesterolemia may also account for erectile impairment based on impaired endothelial NOS dependent mechanisms. The belief that hypogonadism predisposes to erectile dysfunction is supported by recent studies showing that the nitric oxide pathway is androgen dependent.[43–45] Aging phenomena correlate with altered nitric oxide synthesis and erectile responses in the rat penis.[53] Radiation effects have been shown to reduce the number of penile NOS containing nerves in the rat, which offers 1 explanation for the deterioration of penile erections following pelvic irradiation in man.[54] Similar changes result following surgical transection of cavernous nerves in rats,[19,55] although regeneration of penile NOS containing nerves coincident with recovery of erectile function in these animals is observed if the cavernous nerve injury is only unilateral.[55]

Further support for the role of nitric oxide in erectile function has come from preclinical studies in rabbits and clinical trials in men with erectile dysfunction in which the intracavernous administration of linsidomine chlorhydrate (SIN-1), a pharmaceutical agent that releases nitric oxide nonenzymatically, was found to induce erectile responses.[56,57] This nitric oxide donor additionally produced no inflammatory or fibrous reactions of the penile erectile tissue and caused no local discomfort following injection in men, suggesting its highly physiological action. However, its pharmacological smooth muscle relaxing therapeutic effects appeared to be less efficacious than other vasoactive agents in current use, such as prostaglandin E1. The future development of increasingly efficacious pharmaceutical agents based on knowledge of the nitric oxide regulatory pathway is eagerly anticipated.

The prominent role of nitric oxide in the physiology of the penis raises the possibility that this chemical influences

other disease entities of the penis other than erectile dysfunction, in which erectile tissue dysfunction is observed. It is postulated that Peyronie's disease, trauma, tumor infiltration, scleroderma and priapism, all of which represent penile end organ dysfunction, involve impaired NOS regulation or dysfunction. The exact pathophysiology of many of these diseases is not completely understood, although histopathologically these diseases commonly exhibit erectile tissue degenerative changes. These changes include decomposition of neuronal elements, smooth muscle cell cytoplasmic vacuolization and endothelial disintegration,[58] which are frequently associated with hypoxia or acidosis. In view of this observation, the pathophysiology of penile end organ dysfunction could involve impaired nitric oxide effects since nitric oxide production critically requires the presence of oxygen and physiological hydrogen ion concentrations.[39] Down regulation of endothelial NOS gene expression by hemodynamic compromise[6] offers another mechanism whereby local phenomena may interfere with protective effects of nitric oxide. A defective nitric oxide-cGMP pathway could also compound pathological changes via blood flow disturbances in the penis, since this pathway is known to deter platelet aggregation and adhesion.[4,59]

Conversely, an excess of nitric oxide could produce pathological changes in the penis, consistent with its cytotoxic potential as a free radical source.[39,60] By interacting with specific molecular targets, nitric oxide is able to damage cells in many ways, such as by inhibiting adenosine triphosphate production, disrupting deoxyribonucleic acid synthesis or inducing direct toxic effects involving mechanisms that remain unclear. While the injurious properties of nitric oxide may be normally opposed by high levels of superoxide dismutase in the penis,[42] any imbalance in this system might well lead to nitric oxide mediated pathological effects. Recent studies in the rat, which show that an inducible NOS can be expressed in corporeal smooth muscle cells,[38] support the conjecture that nitric oxide can be generated in abundance in the penis.

CONCLUSIONS

Nitric oxide is strongly associated with the physiology of the penis, based on mounting basic science and clinical evidence. This chemical is clearly understood to have major importance in mediating penile erections adhering to signal transduction mechanisms in which guanylate cyclase is activated to produce cGMP, an effector of corporeal smooth muscle relaxation. Medical disease or even iatrogenic insults could directly disrupt nitric oxide mediated erectile mechanisms. Local tissue conditions, including hypoxemia and hypercarbia, or even mechanical deformations may lead to erectile dysfunction via impaired NOS regulation. In fact, such impaired regulation of nitric oxide production would offer a pathophysiological mechanism for the intrinsic erectile tissue dysfunction associated with many penile end organ dysfunctions. Alternatively, any situation involving the over production of nitric oxide could merely have deleterious consequences on the basis of nitric oxide cytotoxicity subsequent to its release in excess quantities. Although the general concepts of nitric oxide biology in the penis are known and logically extend from studies of other physiological systems, further research is required to determine the extent to which nitric oxide has a pathogenic role in this organ.

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